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# PRC2 Goes Solo in the *Drosophila* Female Germline

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**Polycomb-group proteins silence gene expression through epigenetic modification of chromatin. In this issue of *Developmental Cell*, Iovino et al. (2013) demonstrate that Polycomb repressive complex 2 (PRC2) is required for maintenance of oocyte fate by repressing expression of two critical targets, *Cyclin E* and *dacapo*, during the early meiotic cycle.**

The developmental journey from undifferentiated germ cell to functional oocyte requires extensive reorganization of the genome to prepare for the complex process of meiosis. Studies over the last 15 years have shown that histone modifications play a key role in preparing chromatin for meiotic recombination and chromosome segregation (Ivanovska and Orr-Weaver, 2006). However, less is known about how chromatin structure within germ cells modifies gene expression to drive the differentiation of the gamete. In this issue of *Developmental Cell*, Iovino and colleagues (2013) define a role for Polycomb repressive complex 2 (PRC2) in the regulation of the early meiotic cycle and the maintenance of the oocyte fate during *Drosophila* oogenesis. The authors demonstrate that PRC2 prevents the oocyte from developing as a polyploid nurse cell by inhibiting transcription of two cell-cycle genes, *cyclin E* (*cycE*) and *dacapo* (*dap*). In contrast, Polycomb repressive complex 1 (PRC1) components are not required for oogenesis. Thus, the female germline represents a rare example of PRC2 acting independently of PRC1 in *Drosophila*.

Now known as important gene silencers in higher eukaryotes, Polycomb group (PcG) genes were first identified as genes necessary for the maintenance of HOX gene silencing in *Drosophila*. While early studies concentrated on PcG regulation of HOX genes, genome-wide analysis indicates that there are hundreds of PcG targets, including cell-cycle and cell-differentiation genes. Polycomb group proteins act in protein complexes. Two major complexes have been extensively studied: PRC1 and PRC2. PRC1 is composed of Polycomb, Polyhomeotic, dRING/Sce, and Psc or Su(z)2, and PRC2 is composed of E(z), Esc or Escl, Su(z)12, and p55. PRC2 trimethylates histone H3 on lysine 27; H3K27me3 is the chromatin mark of a Pc domain. In *Drosophila*, PcG proteins are recruited to DNA by Polycomb response elements (PREs), in which multiple PRE-DNA binding proteins act together to recruit PRC1 and PRC2 by an undefined mechanism. Genome-wide studies on PcG-regulated genes in *Drosophila* have concentrated on those targets that bind both PRC1 and PRC2 components (Schwartz et al., 2006). However, it is clear from other organisms that PRC1 and PRC2 can act separately to control gene expression. In the germline of *Caenorhabditis elegans*, whose genome does not encode components of PRC1, PRC2 acts to inhibit the expression of somatic differentiation programs and is required for both germline formation and maintenance (Patel et al., 2012; Seydoux and Strome, 1999). Furthermore, in mammals it is quite clear that PRC1 and PRC2 can act at discrete targets (reviewed in Simon and Kingston, 2013); one example occurs in the female germline where PRC2 mediates X inactivation independent of PRC1.

In this manuscript, Iovino and colleagues demonstrate that PRC2 controls *Drosophila* oocyte cell-fate determination independent of PRC1. The data to support this conclusion are quite strong. Germline clones of an *E(z)* null mutant, as well as germline RNA interference (RNAi) knockdowns of *E(z)*, result in egg chambers developing without an oocyte. Furthermore, germline clones of an additional PRC2 component, *Su(z)12*, result in early egg chamber degeneration. In contrast, as shown here using germline Polycomb RNAi knockdown and germline clones, and reported in several earlier studies, germline mutants of multiple PRC1 components have no apparent effect on oogenesis and do not cause sterility. Thus, PRC2 has an essential PRC1-independent function in the female germline.

How might E(z) influence the maintenance of oocyte fate? In *Drosophila*, oocyte development takes place within the context of a collection of 16 cells often referred to as an ovarian cyst. While all 16 cells enter meiosis and proceed through premeiotic S phase, only the oocyte, which is located at the center of the cyst, remains in meiosis and develops as the single oocyte. The other 15 cells enter the endocycle and become polyploid nurse cells. The authors show that in *E(z)* germline knockdowns, and in *E(z)* germline clones, the oocyte is initially specified correctly, as indicated by the accumulation of oocyte-specific markers and the restriction of the synaptonemal complex to a single cell at the center of the ovarian cyst. However, this differentiated state is not maintained, and the oocyte enters the endocycle with the nurse cells and becomes polyploid. The polyploidization of the oocyte is accompanied by the loss of oocyte-specific markers. Thus, E(z) is required for the maintenance, but not the initial specification, of the oocyte fate. Intriguingly, the *E(z)* ovarian phenotype is strikingly similar to mutants of *dap*, a p27-like cyclin-dependent kinase inhibitor (Hong et al., 2003;

Narbonne-Reveau and Lilly, 2009). Like *E(z)* mutants, *dap* ovarian cysts contain 16 polyploid nurse cells and no oocyte.

The endocycle is driven by oscillations of CycE/Cdk2 activity. In nurse cells, the oscillation of CycE/Cdk2 activity is at least partially determined by the reciprocal oscillation of the CycE/Cdk2 inhibitor Dap (Hong et al., 2007). In wild-type ovaries, the levels of CycE and Dap are low during a period that starts soon after the ovarian cysts enter the meiotic cycle and ends when the nurse cells enter the endocycle. This developmental window coincides with the specification of the oocyte and the restriction of the meiotic cycle to a single cell. In *E(z)* mutants, CycE and Dap are overexpressed during this critical period. Moreover, the precocious expression of these cell-cycle genes is accompanied by BrdU incorporation, suggesting that cells within *E(z)* ovarian cysts prematurely enter the endocycle. In order to test the model that *CycE* and *dap* are critical targets of E(z) in the female germline, the authors demonstrate that overexpressing *CycE* and *dap* from transgenes during this critical period results in the polyploidization of the oocyte and the loss of the oocyte fate. In contrast, overexpressing *CycE* and *dap* later in oogenesis had no effect on oocyte fate.

Iovino and colleagues go on to show that *CycE* and *dap* are direct targets of PRC2. The authors use chromatin immunoprecipitation followed by quantitative PCR to demonstrate that E(z) and H3K27me3 are localized at the *CycE* and *dap* genes in ovaries. Interestingly, while numerous cell-cycle genes have previously been shown to contain PREs in embryos and larvae, *CycE* and *dap* are not among this collection (Oktaba et al., 2008). Furthermore, neither *CycE* nor *dap* carries the H3K27me3 mark in 4–12 hr embryos. Thus, it is possible that control of *CycE* and *dap* by PRC2 is specific to the female germline.

These data suggest the following model. During the early meiotic cycle, E(z) and potentially other PRC2 components are required to inhibit the expression of *CycE* and *dap* in order to prevent premature entry into the endocycle and the loss of the oocyte fate. E(z) accomplishes this task independent of PRC1. While this model is compelling, it is important to note that the data presented here don't rule out the presence of additional important E(z) targets in the female germline. This work further demonstrates the intimate relationship between cell-cycle control and cell fate during the development of the *Drosophila* oocyte.

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